

# Methyl Jasmonate Promotes the Growth of *Rosa Roxburghii* via Balanceing Pathways referring to Indoleacetic acid (IAA) and Triterpenes.

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## Research Article

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# Abstract

Exogenous phytohormones has become a common strategy to study plant stress and defense, which triggering complex and diverse cross-actions between endogenous phytohormones, which significantly regulate plant growth and defense. In this study, Methyl Jasmonate (MeJA) promoted the most obvious growth phenotype among four different exogenous phytohormones, especially promoted the growth of roots obviously. Subsequently, principal component analysis between physicochemical indexes and MeJA treatment suggested that MeJA promoted the growth of *R. Roxburghii*. MeJA could increase the contents of JA-Ile, IAA and triterpenoids. qPCR results showed that key genes in the JA signal transduction pathway up-regulated trends. And tryptophan synthesis genes, ANTHRANILATE AMINASE  $\alpha 1/\beta 1$  (*ASA1/ASB1*), IAA receptor protein gene *TIR1*, as well as the IAA polar transport protein gene *PIN2* were all up-regulated by MeJA treatment. While, seven key genes of triterpene synthesis pathway performed a similar up-regulated trends. The results of the chord diagram analysis indicated there might be possible mutual regulation relationship among exogenous MeJA, endogenous JA-Ile, IAA and triterpenoids. The correlation analysis among triterpenoids, IAA and JA-Ile suggested that exogenous MeJA could promote the growth of *Rosa Roxburghii* through regulating the JA signal transduction pathway, synthesis and transport of IAA. At the meanwhile, MeJA promoted the synthesis of triterpenoids which could not only regulate the root growth, but also make *Rosa Roxburghii* a certain defense ability. Finally, we speculate that exogenous MeJA pushed endogenous JA-Ile and IAA involved in regulated the metabolism of triterpenoids, they together regulated the positive growth of plants.

## 1 Introduction

Methyl Jasmonate (MeJA) is also a well-known mediator of biotic and abiotic stress responses, it can significantly and broadly regulate the synthesis of defensive metabolites and initiate the expression of genes associated with systemically acquired and localized resistance (Wasternack et al., 2013; Zhu, 2014). The jasmonin pathway is an important signaling pathway in plants. The biologically active jasmonic acid derivative jasmonoyl-isoleucine (JA-Ile) could binds to the F-box protein COI1, resulting in the protein ubiquitination of JAZ protein, the negative regulator of the jasmonin pathway, and release transcription activators, thereby activating the expressions of functional genes downstream. Jasmonin's functions involved in the regulation of plant growth and development such as root growth, plant fertility, tuber formation, fruit ripening and senescence and other life activities, even play important roles in plant biotic and abiotic stress (Pollier et al., 2013; Wasternack & Hause, 2013). Meanwhile, MeJA could promote the accumulation of other phytohormones (Bai et al., 2019; Garza et al., 2009; Post et al., 2012). Crosstalk usually exists between phytohormones, in some cases the crosstalk was controlled by those shared components during signal transduction pathways, which reflected the regulation of the synthesis of another by a given hormone (Du et al., 2013; Gutierrez et al., 2012; Hentrich et al., 2013). For example, MeJA could regulate the expression of ANTHRANILATE AMINASE  $\alpha 1/\beta 1$  (*ASA1/ASB1*) to achieve metabolic effects on the tryptophan pathway (Dombrecht et al., 2007; Sun et al., 2009), and promote the accumulation of IAA by increasing tryptophan, the formation of IAA precursor. And MeJA could even

directly regulate the *YUCCA* gene in the IAA synthesis pathway to regulating the synthesis of IAA in roots (Hentrich et al., 2013). MeJA could also affect the polar transport of auxin and promote the function of auxin, such as, MeJA could participate in the transport of auxin by affecting the distribution and expression of the polar auxin transporter genes *PIN2* (Sun et al., 2011). Otherwise, MeJA and IAA could also interact through the action of auxin response factor (ARF) to regulate the transcription of downstream auxin-dependent genes (Fattorini et al., 2018). Through these ways, but not only these, MeJA closely cooperates with IAA in regulating the formation and growth of adventitious roots formation (Gutjahr et al., 2005; Sharma et al., 2022; Wang et al., 2022). IAA in plants could affect the early division of plants, root elongation and the formation of tissues (Rui et al., 2020). MeJA could regulates a variety of secondary metabolites, especially terpenoids (Rui et al., 2020; Sirhindi et al., 2020). MeJA could not only significantly up-regulated the transcription level of genes in MVA pathway, MEP pathway and the triterpenoid synthesis pathway, but also regulated the synthesis of triterpenes through the endoplasmic reticulum-related degradation quality control system (Bai et al., 2019; Pollier et al., 2013), thereby promoting the accumulation of triterpenoids in plants and achieving plant resistance to adverse environments.

In order to adapt to different environmental niches, plants had evolved the ability to produce large, diverse, and specific secondary metabolites, at the meanwhile, specific metabolites of plants often have certain ecological functions and mediate the interaction between plants and the environment (Chae et al., 2014; Huang et al., 2019). Triterpenes are one of plant-specific metabolites, which are almost the largest and most structurally diverse natural production family, with plant defense and signal transduction functions (Augustin et al., 2011; Papadopoulou et al., 1999). Triterpenes are widely distributed in various parts of plants and play an important role during plant growth, such as, the expression of triterpenes in roots can trigger the "super-hairy" root phenotype (Kemen et al., 2014), and the marginal cells of alfalfa heel secrete large amounts of triterpenoids to protect the fragile root cap and ensure root development and growth (Field et al., 2008; Watson et al., 2015). Triterpenes in stems can effectively protect plants against dehydration, they defended against potential herbivore damage together with triterpenes secreted in leaves (Thimmappa et al., 2014). Compared with traditional chemical pesticides, triterpenes are greener and healthier as pesticides. At present, many triterpenoids containing C-28 carboxyl groups have been developed and applied as biological pesticides (Tian et al., 2021). In terms of defense function, the synthesis of triterpenes can increase the tolerance of plants to external stress and alleviate the damage of UV-B to plants (Jiang et al., 2020). In addition to the defense function, triterpenoids also have good antibacterial activity. In plants such as *Arabidopsis* (Huang et al., 2019) and *Schisandra* (Jacoby et al., 2020), triterpenoids could play as signal molecules, nutrient sources, antibiotics, etc., regulating endophytes which are beneficial to growth (You et al., 2021), and promoting plant growth and metabolism (van Damme et al., 2006).

*Rosa Roxburghii* (also named Cili in Chinese) is a unique medicinal and edible plant that grows in southwest China, which has a long history and belongs to the Rosaceae family (Xu et al., 2019). It was first recorded in the Compendium of Materia Medica. People make use of its advantages of rich in vitamin C and antioxidant enzymes like SOD and POD (Liu et al., 2016). The fruit of *R. Roxburghii* were

mass-produced as functional foods, such as Cili wine, preserved fruits and juice, etc. With the deepening of research, the mysterious veil of *R. Roxburghii* has been unraveled: *R. Roxburghii* was not only rich in vitamin C, but also rich in bioactive secondary metabolites, especially the specific triterpenoids including kajiichigoside F1, roxburic acid, rosamultin, pomonic acid, and tormentic acid, as well as Oleanolic acid, etc (Xu et al., 2019). These triterpenoids rich in active hydroxyl and carboxyl groups make the excellent performance of *R. Roxburghii* as a functional food in anti-tumor, anti-oxidation, anti-virus and radiation protection (Alkhatib, 2020; Jo et al., 2020; Wang et al., 2020; Xu et al., 2017; Xu et al., 2020; Xu et al., 2018). Even Academician Zhong Nanshan proposed that Cili has high nutritional value and health care effects to play a positive effect on the prevention of Corona Virus Disease 2019, at the Guizhou Cili Industry Development Forum held in Guangzhou, On April 28, 2020.

In this study, *R. Roxburghii* were treated with different hormones, and various growth trends were observed, which MeJA promoted the most obvious growth phenotype among them. In order to explore the possible reasons how MeJA promotes the growth of *R. Roxburghii* seedlings, we monitored some physicochemical indexes, including contents of MAD, peroxidases, soluble sugar, soluble protein and proline. We found that the contents of IAA, JA-Ile and triterpenoids in *R. Roxburghii* were significantly increased after MeJA treated. In order to found out the possible mutual regulation relationship among MeJA, IAA and triterpenes, the JA receptor protein gene *COI1*, and one of the SCF<sup>COI1</sup> complex proteins gene *ASK1*, the IAA synthesis pathway genes ANTHRANILATE AMINOTRANSFERASE  $\alpha 1/\beta 1$  (*ASA1/ASB1*), The IAA receptor protein gene *TIR1*, the IAA polar transport protein gene *PIN2* and key genes in the triterpene synthesis pathway including 3-hydroxy-3-methylglutaryl coenzyme-A reductase (*HMGR*), 1-Deoxy-D-xylulose 5-phosphate synthase (*DXS*), 1-Deoxy-D-xylulose 5-phosphate reductoisomerase (*DXR*), 1-hydroxy-2-methyl-2-(E)-butenyl-4-diphosphate reductase (*HDR*), squalene synthetase (*SQS*), squalene epoxidase (*SQE*) and amyrin synthetase (*AS*) were quantified by qPCR. Finally, the qPCR results were consistent with our hypothesis. Our experimental results preliminarily explain that MeJA promoted the JA signal transduction pathway, as well as the synthesis and transport of IAA. While, MeJA could also accelerated the accumulation of triterpenoids which could not only regulate the root growth of *R. Roxburghii* but also endow *R. Roxburghii* with certain defense ability. In addition, we speculate that JA and IAA might also be involved in affecting the synthesis and metabolism of triterpenes, they promoted the growth of plants together, however, all these changes might cause by the only variate value, exogenous MeJA.

## 2. Materials And Methods

### 2.1 Materials and reagent

*R. Roxburghii* seeds were purchased from Guizhou, Qiannan, China. Hoagland's medium was purchased from Coolaber, Beijing. Methyl jasmonate was purchased from Sigma-Aldrich. GA<sub>3</sub>, IAA and ABA were purchased from Songong, Shanghai. Ethephon was purchased from Sigma-Aldrich. JA-Ile was purchased from MKBio, Shanghai. Mass spectrometry methanol was purchased from J&K Chemical LTD, Beijing.

The purified water used in mass spectrometry was purchased from Wahaha Purified Water Company. Oleanolic acid and ursolic acid were purchased from Sigma-Aldrich. The roxburic acid, kajiichigoside F1, euscaphic acid and pomolic acid were separated and purified by the Key Laboratory of Forest Plant Ecology of the Ministry of Education, Northeast Forestry University.

## 2.2 Plant cultivation and treatment

*R. Roxburghii* seeds germinate in the soil. The seedling age of 30-day *R. Roxburghii* seedlings were washed with distilled water to remove the soil, and transferred to Hoagland's nutrient solution for 7 days, then treated with phytohormones.

Four hormones were added to Hoagland's medium as 100Mm MeJA, 50 mg/L ethephon ETH, and 200  $\mu$ M GA3, 100 $\mu$ M ABA. Each treatment consisted of five replicates, and each experiment were repeated three times. Observations and analysis on the biomass production, and triterpenoid contents.

## 2.3 Determination of total triterpenoid contents

Determine the total triterpene content by modifying the method described by Madhuri Vaghela et al. In short, mix each extract (100 $\mu$ L) with vanillin glacial acetic acid (5% w/v, 150 $\mu$ L) and (300 $\mu$ L) perchloric acid. All reaction mixtures were incubated for 20 min at 60 C, and turned them into ice for 15 min. Then, added 2.5 mL glacial acetic acid and stand for 10 minutes, the absorbance was examined at 545 nm. The calibration equation for ursolic acid, as a standard, was  $Y = 7.0534X + 0.0228$  ( $R^2 = 0.9941$ ), (Y was the absorbance value, X mean the content of ursolic acid (mg)). Calculated each average value and standard deviation of the total triterpene content at each growth stage.

## 2.4 Determination of physicochemical indexes

Malondialdehyde (MDA) assay kit, Superoxide Dismutase (SOD) typed assay kit, Peroxidase assay kit, Catalase (CAT) assay kit, Proline assay kit, Plant soluble sugar content test kit, Total protein quantitative assay kit were purchased from Nanjing Jianchenng Bioengineering Institute. All the physicochemical index was determined follow the instruction manual of kits separately. Each sample was tested in triplicate.

## 2.5 Extraction and quantification of roxburic triterpenoids by HPLC-MS/MS

Different *R. Roxburghii* plants were subjected to metabolite analyses. Each sample was accurately weighed to 0.1 g, 5 mL of 80% ethanol and aqueous solution were used for extraction, the ratio of solid to solvent was 1:50 (w/v), and ultrasonic extraction was performed at room temperature with a power of 80 W for 30 min. After centrifugation at 10,000 for 5 min, the supernatant was collected and filtered using a filter with 0.22  $\mu$ m PTFE membrane. Samples were diluted for UPLC-MS/MS analysis.

Thermo Fisher Orbitrap Q Exactive Plus Mass Spectrometer was used for analysis. The ion source was ionization polarity: ESI<sup>-</sup>&ESI<sup>+</sup> in positive or negative ion mode for all analytes. The data system was

Compound Data software version 3.3 (Thermo). Separations were performed using the Hyperil Gold (100×2.1 mm, 1.9 µm) column. The flow rate of the mobile phase was 0.35 mL/min. The mobile phase was a linear gradient of 0.1% (v/v) aqueous formic acid (A) and Methanol (B): 0–1.0 min, 98% A (v/v); 1.0–9.0 min, 2% A (v/v); 9.0–12.0 min, 2% A (v/v); 12.0–12.10 min, 98% A (v/v); 12.10–15.0 min, 98% A (v/v). The column was reconditioned for at least 3 min before every injection. 2 µL sample was injected for each run. The optimum operating ESI conditions were: Aux gas temperature: 350°C, sheath gas: 40 arb, Aux gas: 10 arb, capillary temperature: 320°C, S-lens RF level: 60. The spray voltages were optimized to 3800/3000 V (+/-).

## 2.6 RNA extraction and cDNA synthesis

Multiple plants of controls and treated samples were frozen in liquid nitrogen then stored at – 80°C before RNA extraction. Total RNA was extracted from plants (20 mg) using Plant RNA Mini-Extraction Kit (Omega Bio-Tek, USA) according to the manufacturer's protocol. Nano Photometer™ (UV/Vis Spectrophotometer, Implen, Germany) and agarose gel electrophoresis were used for qualitative and quantitative estimation of RNA. cDNA synthesis was carried out by PrimeScript 1st strand cDNA Synthesis Kit (TaKaRa, Japan) from 1 µg RNA of samples according to the manufacturer protocol.

## 2.7 Gene expression analysis by qPCR

Gene differential expression of the *ASA1*, *ASB1*, *TIR1*, *PIN2*, as well as six key genes involved in triterpenoid biosynthesis, *SQS*, *SQE*, *AS*, *DXP*, *DXR* and *HMGR* was done. Primers were listed in supplemental materials Table S1. Real-time PCR was performed on the Light Cycler 96 system (Roche, USA) using TB Green Premix Ex Taq (Tli RnaseH Plus) (TaKaRa, Japan). The reaction mixture consisted of 10 µL of TB Green Premix Ex Taq II (2X), 2 µL of primers (5 µmol), 2 µL of template (diluted cDNA), and DEPC water was used to make up to 20 µL. The cycling parameters were: initial denaturation for 30 s at 95 °C; 38 cycles of 5 s at 95 °C, 30 s at 55 °C followed by melting cycle from 70 to 95 °C. Data from qRT-PCR amplification were calculated using the comparative Ct ( $2^{-\Delta\Delta Ct}$ ) method, where the cycle thresholds Ct values of the calibrator and the samples were normalized to the endogenous housekeeping gene 5.8s rRNA. Three technical replicate and thrice repeated analysis were performed.

## 2.8 Method validation

The stability of the method was estimated via RSD value which the *R. roxburghii* samples were placed at room temperature for 2h, 4h, 8h, 12h, 24h and 48h, respectively before HPLC analysis, which was repeated six times. The precision of the method was estimated by calculating the RSD value of 6 times analysis. The recovery of the method was evaluated by analyzing six samples added different concentration standard solutions at 12.5, 25 and 50 µg/mL. All results of method validation are showed Supplemental Table S2

## 2.9 Statistical analysis

The SPSS version 21 (IBM Corp. Chicago, USA) was used for data analysis. Data were presented as the standard deviation of the mean (mean ± SD). The statistical significance of the values was carried out

using one-way analysis of variance (ANOVA) with Duncan's multiple range test ( $p < 0.05$ ). In order to explore the correlation between triterpenoids, MeJA and IAA, spearman analysis was carried out. The chord diagram and principal component analysis (PCA) were carried out by Origin 2021b (OriginLab Corporation, Northampton, MA, USA) to analyze the integrated responses of *R. Roxburghii* to MeJA treatment.

## 3 Results

### 3.1 Exogenous phytohormones regulated the growth of *R. Roxburghii*.

Triterpenoids were reported be sensitive to drought, in order to getting a correct result, *R. Roxburghii* were cultured in a water-medium in this study, then treated with different concentrations of exogenous phytohormones, the phenotype of *R. Roxburghii* growth were changes differently (Fig. 1a). MeJA had a relatively obvious promoting effect on the growth of *R. Roxburghii*, and there was no obvious hysteresis in the roots and the up-ground parts. GA3 and ABA Exogenous hormones had obvious effects on the growth of *R. Roxburghii* seedlings, but the growth of the up-ground was not very obvious. Among the four hormones, only ETH did not significantly promote the growth of *R. Roxburghii*, but the effect of ETH made the roots thicker, which might be related to the ETH could promote the lignin accumulation. Based on the observed phenotypes, we measured the growth of roots and the up-ground parts of *R. Roxburghii* seedlings, and the results were consistent with the phenotype (Fig. 1).

### 3.2 MeJA promoted the growth and metabolism of *R. Roxburghii*.

We kept on monitoring the MDA, peroxidase, soluble sugar, soluble protein and proline in *R. Roxburghii* roots. The detection results were shown in Fig. 2a-g. Analysis of the physical and chemical indexes in the *R. Roxburghii* roots suggested that MeJA cause no strong changes in MDA, SOD and CAT in the *R. Roxburghii* roots, which implied that low concentrations of MeJA could be beneficial to *R. Roxburghii* growth. The increase of POD value indicated that MeJA not only cause little fluctuate of oxidative stress in root cells, but also delayed the senescence of cells and promote cell viability. The increase of soluble protein and free proline in Fig. 2e and Fig. 2g also illustrated that the metabolic capacity of *R. Roxburghii* root cells were stronger after treated with MeJA. However, the rapid metabolism of cells does not mean the biomass increase or elongation of roots. Subsequently, the increase of soluble sugars in Fig. 2f indicated that MeJA could promote the root biomass by increasing the accumulation of nutrients in *R. Roxburghii* and enhance the metabolic activity of root cells to make sure the growth of *R. Roxburghii* roots at the same time.

According to Fig. 3h, principal component analysis (PCA) showed represented 74.33% and 14.4% variation, at the first and second axis, of total seven parameters, the total variation of two axes were

88.73%. The MDA, proline and soluble sugar appeared in the first quadrant and performed a negative correlation with soluble protein and CAT. The CAT appeared in the second quadrant and had a negative connection with other parameters. All treated samples were heterogeneous in PCA model, and could be clustered into eight groups meeting eight MeJA treatments.

### **3.3 Exogenous MeJA change the contents of metabolites in *R. Roxburghii*.**

After MeJA treatment, we monitored the changes of several typical hormones in *R. Roxburghii*. The contents of gibberellin and abscisic acid were relatively stable under the exogenous MeJA treatment (Supplemental Fig S1 and Supplemental Fig S2). However, it was found that the content of jasmonin in *R. Roxburghii* decreased slightly, but not significantly. Increased levels of auxin and jasmonin derivatives occurred as showing in Fig. 3a and Fig. 3b.

In the subsequent study, we detected the contents of triterpenes in *R. Roxburghii*. Results showed that the content of triterpenes in the MeJA treatment group increased slowly from 0 day to the eighth day, until the twelfth day, triterpenoids began to accumulate in large quantities, compared with the control group (Supplemental Fig S4). The growth of *R. Roxburghii* supplied more cell units that could join in triterpenes synthesis, till *R. Roxburghii* was treated with MeJA for two weeks. In addition, we also monitored the contents of several special *R. Roxburghii* triterpenes in the root. The results showed that roxburic acid, kajiichigoside F1, euscaphic acid and pomolic acid were all increased in addition to oleanolic acid (Fig. 3c). In other words, the incremental part of the total content of triterpenes in *R. Roxburghii* was mainly formed by the up-regulated synthesis of ursane-type triterpenes. The differential version of five special triterpenoid in *R. Roxburghii* suggest that MeJA might participate in or regulate the biosynthesis of triterpenoids in *R. Roxburghii*. Correlation analysis based on Spearman analysis was performed on the five specific triterpenes, IAA and JA-Ile, and it showed that the synthesis of triterpenoids was positively correlated with both two up-related hormones in *R. Roxburghii* (Fig. 3d).

### **3.4 Exogenous MeJA regulated the JA signal transduction pathway and IAA related pathway of *R. Roxburghii*.**

There are many hormones that regulate plant growth, Jasmine and auxin were the most important, especially for the regulation of plant roots. qPCR analysis was used to find out whether the growth of *R. Roxburghii* was related to Jasmine and auxin. After MeJA treatment, the content of JA-Ile and IAA in *R. Roxburghii* increased (Fig. 3), we speculated that the increase of JA-Ile and IAA might be related to the exogenous MeJA. COI1, as the receptor of JA-Ile, and ASK1 which could form complexes with COI1, were chosen to showed the expression of JA signal transduction pathway. Results in Fig. 4a and Fig. 4b suggested that not only the JA-Ile receptor was up-regulated while the accelerate of JA-Ile, but also the SCF<sup>COI1</sup> complexes. It means the classic JA signal is triggered. It has been reported that obvious crosstalks were found between MeJA and indoleacetic acid (IAA) (Marasek-Ciolakowska et al., 2020).

When IAA was synthesized in plants, it immediately bound to the IAA receptor (TIR1) on SCF<sup>TIR</sup> complex and forms the SCFTIR1-Aux/IAA complex and initiate the subsequent transcription process (Ma et al., 2019). In the qPCR results, *ASA1*, *ASB1*, *TIR1* and *PIN2* had similar change patterns, which indicated that MeJA up-regulated genes related to IAA synthesis (*ASA1*, *ASB1*, *TIR1*), as well as promoted the expression level of IAA polarity transporter genes *PIN2* (Fig. 4c-4f). These results revealed the results that MeJA promoted the growth of *R. Roxburghii* in Fig. 1.

### 3.5 Exogenous MeJA regulated gene differential expressions in triterpenoid synthesis of *R. Roxburghii*.

Figure 5 shows the transcriptional level variation of several key genes related to the triterpenoid synthesis pathway that currently reported already. The expression of the key limiting enzyme in the MVA pathway, *HMGR*, was up-regulated after the treatment of MeJA (Fig. 5a). *HMGR* plays an important role as the first limiting enzyme in the MVA pathway, controlling the reaction speed of the MVA pathway. The synthesis of IPP and DMAPP, so as the final products of the MVA pathway, could be accelerated, when the expression of *HMGR* is up-regulated. Then, analysis of three key enzyme genes in the MEP pathway showed that the expression levels of *DXS*, *DHR* and *HDR* were rising first and then falling, they reached the peak at 24 hours (Fig. 5b-5d). It has been reported that MeJA could regulate the genes of the MEP pathway. This view was confirmed again during our research. Finally, qPCR analysis of *SQS*, *SQE* and *AS* showed that these three key genes in the downstream of pentacyclic triterpenoid synthesis pathway could respond well to the regulation of MeJA. The expression patterns of *SQS*, *SQE* and *AS* were similar, they all showed a trend of increasing at first and then decreasing (Fig. 5e-5g), which indicates that these three genes have similar patterns in response to MeJA. However, there were obvious differences in the order of response to MeJA, the time of begin to increase, the time of reach the peak and when start to decline were significantly different among them. At the meanwhile, this also implied that MeJA regulated the triterpene synthesis genes does not occur simultaneously, there might be any other regulatory mechanisms. Our results above indicated MeJA could up-regulate the expressions of the triterpenoid synthesis genes in *R. Roxburghii*, then promoted triterpenoid synthesis at the transcriptional level.

## 4 Discussion

Plant hormones could regulate the secondary metabolite synthesis through a variety of molecular synthesis, signal transduction and interaction factors. We found that the contents of JA-Ile and IAA in *R. Roxburghii* were both significantly changed after treated with MeJA. It is well known that IAA plays a crucial part of promoting plant growth, especially in the early stage of plant (Cuesta et al., 2013). The root system of plants was inherently highly plastic and influenced by both exogenous and endogenous factors on its structure changes (Roychoudhry et al., 2017). Recently, Roychoudhry, et al. reported that IAA regulated the development of main root and lateral root, then promoted plants better growth (Roychoudhry et al., 2022). Here, based on Fig. 3d, we speculate that the underlying cause of the differential accumulation changes in triterpenoid and the growth may be related to IAA. The qPCR results

of key genes in the IAA synthesis pathway genes *ASA1*, *ASB1*, IAA receptor protein gene *TIR1*, IAA polar transport protein gene *PIN2* and triterpenoid synthesis pathway showed that the expression levels of *ASA1* and *ASB1* genes in the roots of *R. Roxburghii* showed an upward trend, which mean that the synthesis of IAA may be facilitated by sufficient precursor. At the same time, the high expression of *TIR1* gene indicated that the accumulation of IAA increased indeed. The up-regulation of *PIN2* was consistent with the reported conclusion that MeJA could promote the polar transport of IAA. All results above showed that MeJA could promote the synthesis and transport of IAA, and promote the growth of *R. Roxburghii* seedlings indirectly. In addition, there were some reports on the auxin regulated secondary metabolites, for example, IAA could promote the secondary metabolism of *Panax ginseng*, *Morus alba L.* and *Catharanthus roseus*, increase the accumulations of ginsenoside, rutin and alkaloids (Berendsen et al., 2012; Lee et al., 2011; Pasquali et al., 1992). Hence, we speculate that IAA might be also involved in regulating the synthesis of triterpenes in *R. Roxburghii* (Fig. 6D pathway).

There were varieties of specific triterpenes in *R. Roxburghii* including roxburic acid, kajiichigoside F1, euscaphic acid, Oleanolic acid and pomonic acid, has been isolated and purified, *R. Roxburghii* triterpenes were widely distributed and its fruits were recommended as a functional food to prevent COVID-19. *R. Roxburghii* received more attention from researchers in recent years because of their great bioactivities in antiviral, anti-tumor, anti-inflammatory and immunity. There are many cross-links between synthetic pathways in plants. Secondary metabolic pathways could produce precursors of various hormones, such as auxin, gibberellin, abscisic acid, which are synthesized by the isoprenoid pathway. There are common upstream synthetic pathways: MVA pathway and MEP pathway among these pathways and triterpene synthesis pathways, and they share the same precursors IPP and DMAPP. In nature, the ecological niches are diversity, so the diversification of plant metabolism is promoted (Huang et al., 2019). In order to maintaining the growth of plants, specific secondary metabolites usually perform specific ecological functions in different plant species (Tu et al., 2020). These specific triterpenes of *R. Roxburghii* may play some important roles in regulating the growth of *R. Roxburghii*. At this time, adding exogenous hormones to plants could affect the metabolism of plants. In our study, the significantly increased contents of endogenous hormones and triterpenes in MeJA-treated *R. Roxburghii* verified our hypothesis that MeJA may regulate the growth of *R. Roxburghii* by balancing the pathway involved of JA, IAA and triterpenoids (Fig. 6.).

It was reported that MeJA could not only regulate triterpenoid skeleton synthesis genes, but also those terpene modification genes, such as, cytochrome P450 enzymes and glycosyltransferases which make triterpenoid family the most diversification of configuration (Caretto et al., 2011; Kim et al., 2011; Zeng et al., 2018). As well as a key point of this study, the contents of ursane-type triterpenoids changed significantly, and triterpenes with more hydroxyl sites, like roxburic acid, kajiichigoside F1, euscaphic acid and pomonic acid accumulated in *R. Roxburghii* under the regulation of MeJA (Fig. 4b). We speculated that MeJA could specifically regulate the synthesis of triterpenoids in *R. Roxburghii* precisely, so as to adapt to the changes from the growth cycle and environment surrounding. Cytochrome P450, as an important modification enzyme plays a decisive role in realizing the diversification of triterpenes in plants. Subsequently, our research will focus on studying the P450 enzymes in *R. Roxburghii*, aiming to

clarify the further scientific mechanism about how MeJA promotes the growth of *R. Roxburghii* seedlings. Our research could promote more understanding of the formation and specific accumulation of triterpenoids that regulate by MeJA on the molecular level, and also developed the application value of phytohormones in the field of promoting the accumulation of plant secondary metabolites, finally achieving more effective acquisition of high-value plant secondary metabolite resources.

## Declarations

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### Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

### Author Contributions

Yu-jie Fu, Su Zhang and Yuan Ning contributed to the study conception and design. Material preparation, data collection and analysis were performed by Yuan Ning, Yun-kun Ding and Yuan-hang Chang. The first draft of the manuscript was written by Yuan Ning, Yun-kun Ding and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript

### Data Availability Statement

All data generated or analyzed during this study are included in this published article and its supplementary information files.

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## Figures

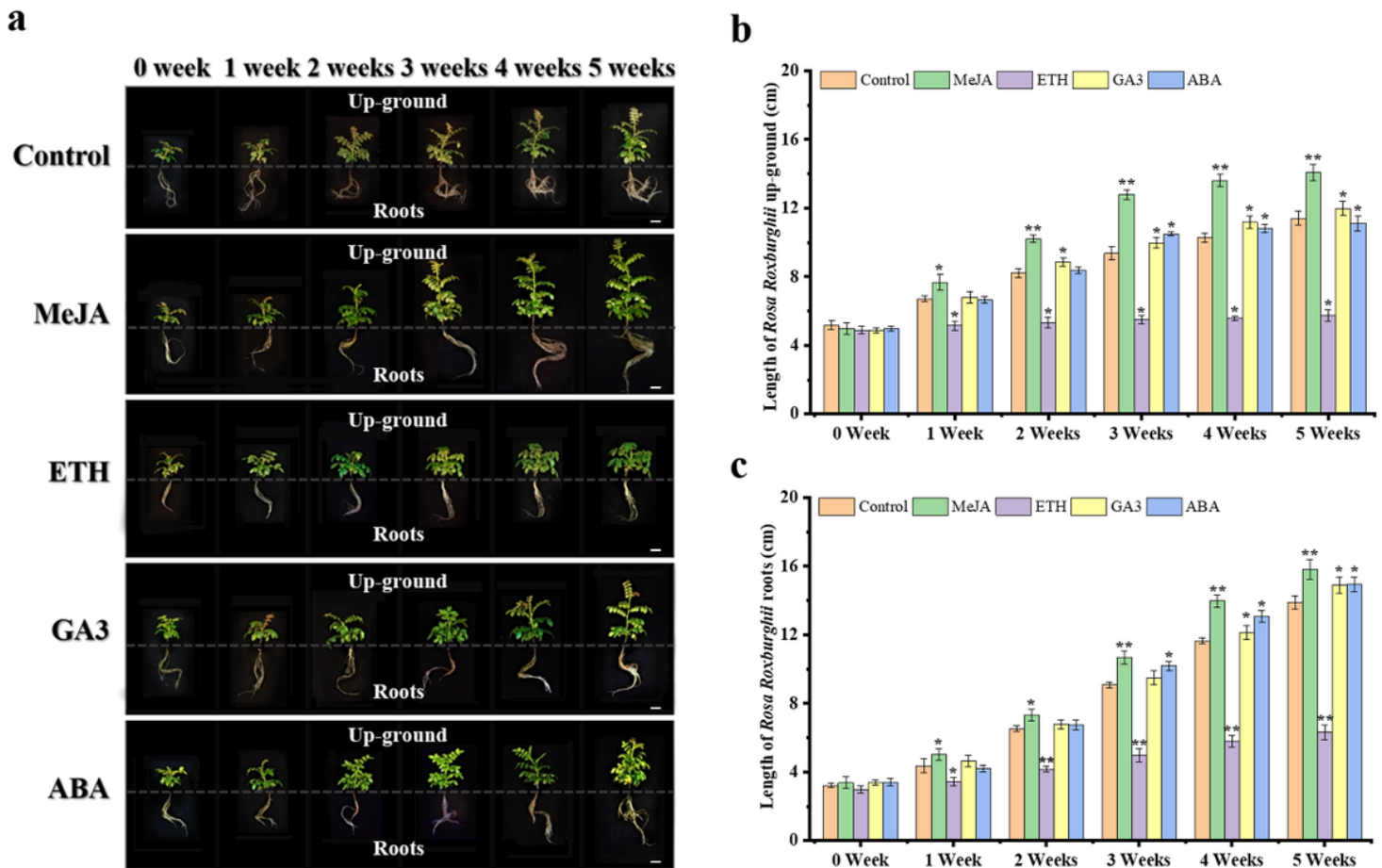
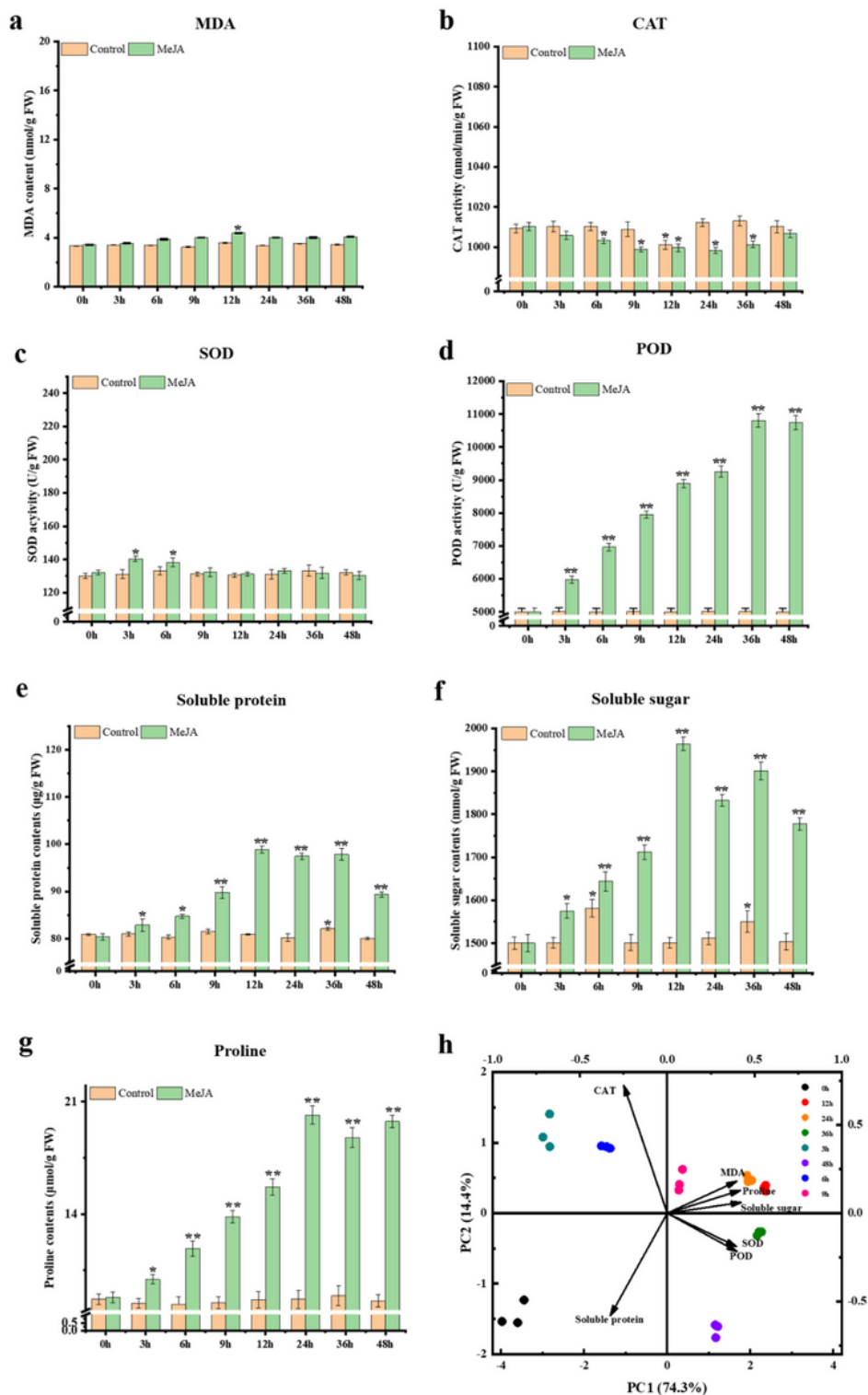


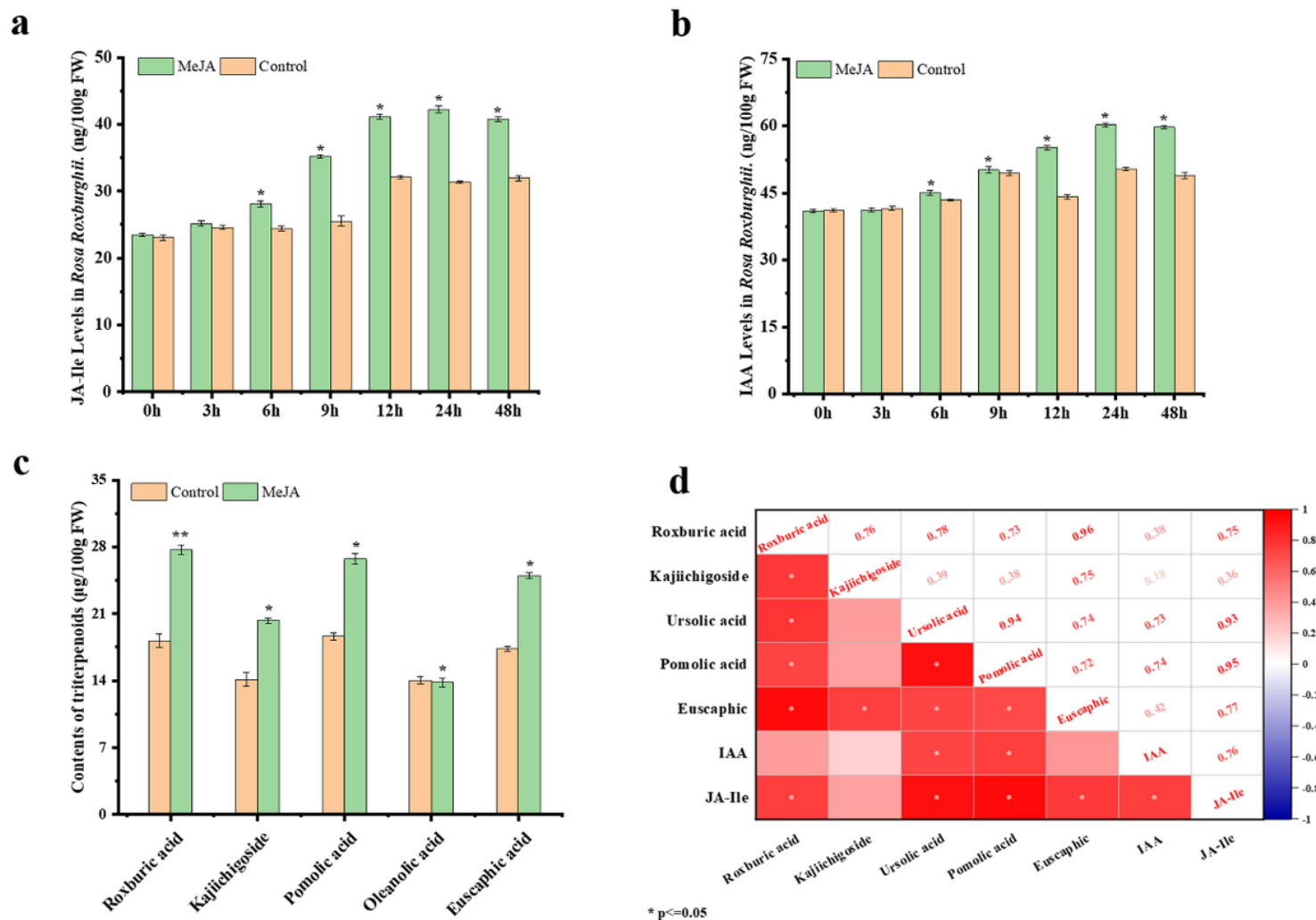
Figure 1

**Phytohormones effected the growth of *R. Roxburghii*** (a): Phenotypes of plants; (b): Growth of up-ground treated with phytohormones; (c): Growth of roots treated with phytohormones. “\*” means  $p < 0.05$  phytohormones treatment groups vs control. “\*\*” means  $p < 0.01$  phytohormones treatment groups vs control. “-” in fig 1(a) stand for 1 centimeter.



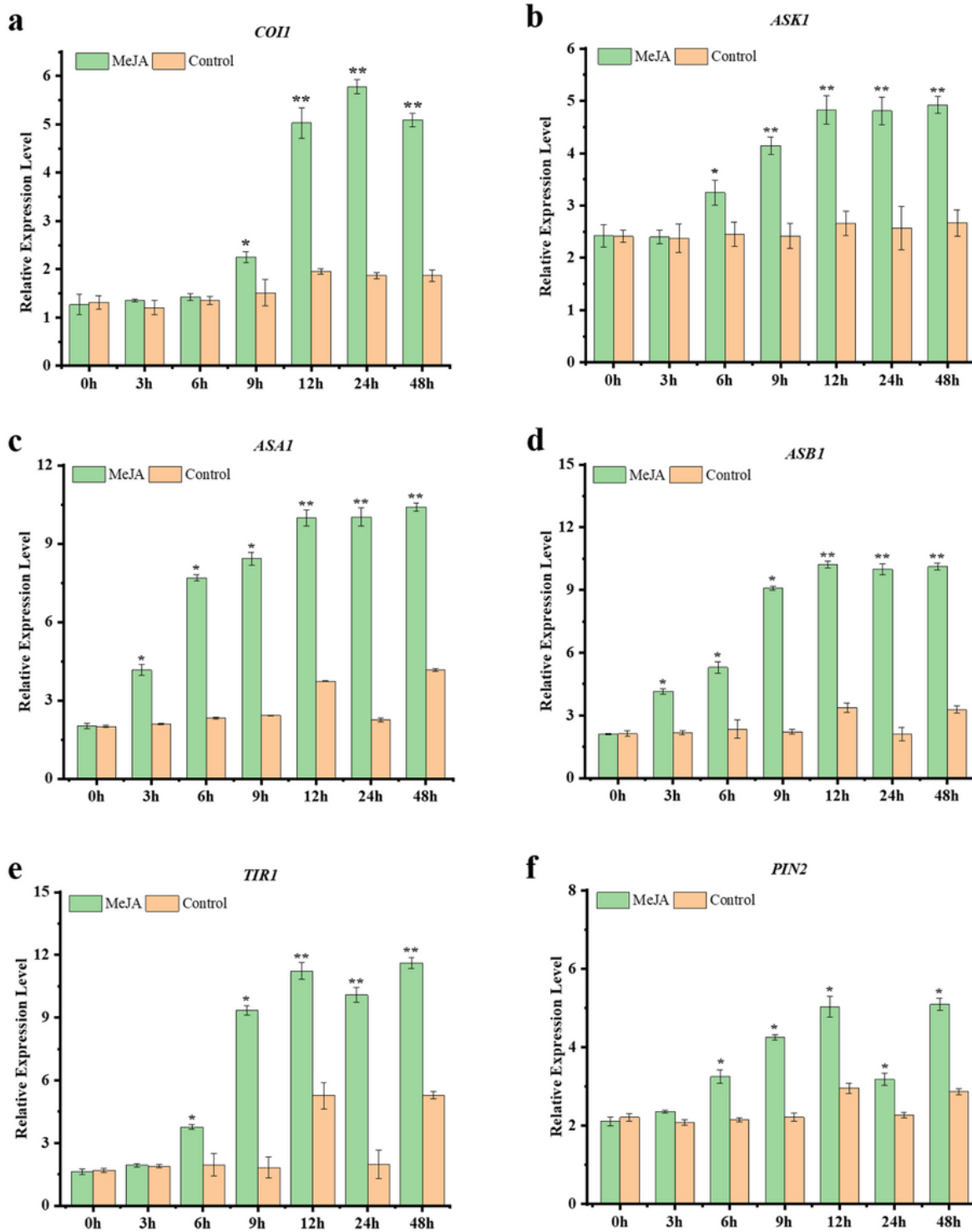
**Figure 2**

**MeJA promoted the metabolite of *R. Roxburghii* through oxygen reactive and nutrient accumulation(a):** MDA content. (b): CAT activity. (c): SOD activity. (d): POD activity. (e): Soluble protein content. (f): Soluble sugar content. (g): Proline content. (h): Principal component analysis for showing the correlation between the MeJA treatment and the physicochemical indexes. “\*” means  $p < 0.05$  MeJA treatment groups vs control. “\*\*” means  $p < 0.01$  MeJA treatment groups vs control.



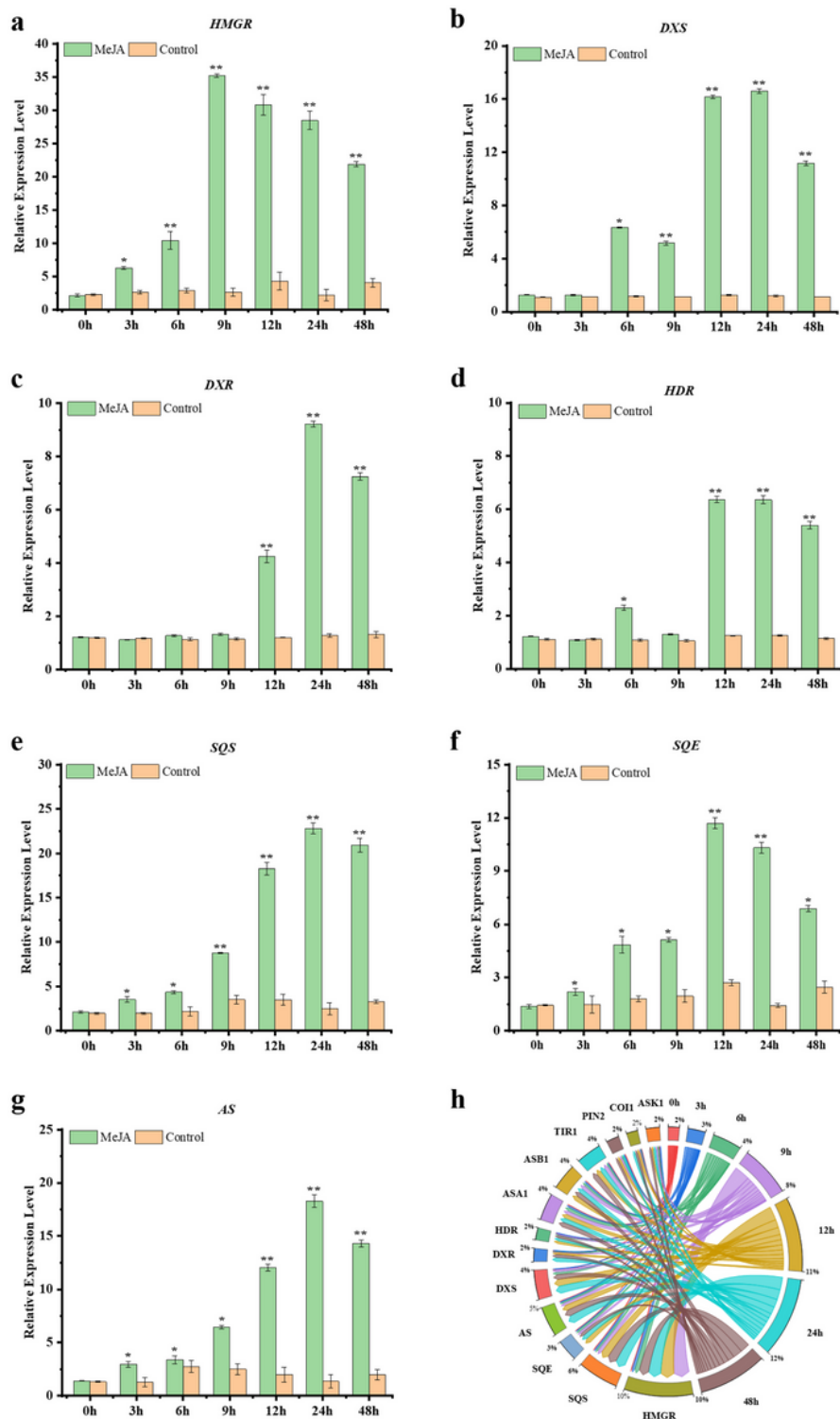
**Figure 3**

**Metabolites changes in *R. Roxburghii*** (a): JA-Ile levels in *R. Roxburghii*. (b): IAA levels in *R. Roxburghii*. (c): Contents of special triterpenes of *R. Roxburghii*. (d): Correlation analysis between endogenous hormones and triterpenoids. “\*” means  $p < 0.05$  MeJA treatment groups vs control. “\*\*” means  $p < 0.01$  MeJA treatment groups vs control.



**Figure 4**

**Relative expression levels of genes related to JA and IAA in *R. Roxburghii*** (a): Relative expression levels of *COI1*. (b): Relative expression levels of *ASK1*. (c): Relative expression levels of *ASA1*. (d): Relative expression levels of *ASB1*. (e): Relative expression levels of *TIR1*. (f): Relative expression levels of *PIN2*. “\*” means  $p < 0.05$  MeJA treatment groups vs control. “\*\*” means  $p < 0.01$  MeJA treatment groups vs control.



**Figure 5**

**MeJA regulated genes differential expressions of triterpenoids synthesis pathway in *R. Roxburghii*(a):** Relative expression levels of *HMGR*. (b): Relative expression levels of *DXS*. (c): Relative expression levels of *DXR*. (d): Relative expression levels of *HDR*. (e): Relative expression levels of *SQS*. (f): Relative expression levels of *SQE*. (g): Relative expression levels of *AS*. (h): Chord diagram between MeJA

treatment and differential expression genes “\*” means  $p < 0.05$  MeJA treatment groups vs control. “\*\*” means  $p < 0.01$  MeJA treatment groups vs control.

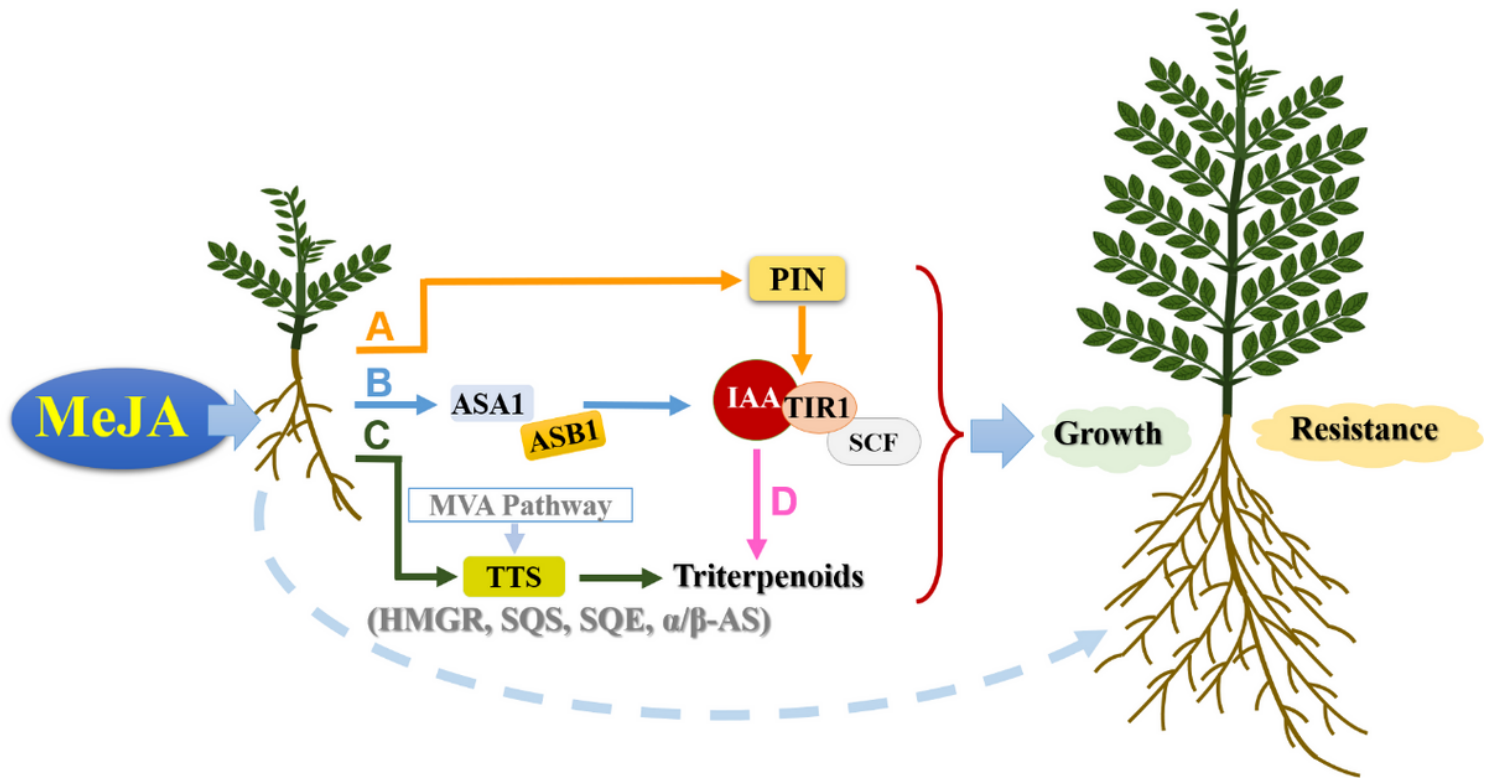


Figure 6

**MeJA promotes the growth of *Rosa roxburghii* via balancing multiple pathways referring to IAA and Triterpenes**  
 A pathway: MeJA promoted the transport of IAA; B pathway: MeJA promoted the synthesis of IAA; C pathway: MeJA promoted the synthesis of triterpenes; D pathway: IAA might promote the synthesis of triterpenes.

## Supplementary Files

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